Dear Sir,

Human RT-4 bladder carcinoma is highly metastatic in nude mice and comparable to ras-H-transformed RT-4 when orthotopically implanted as histologically intact tissue.

It has been estimated that 5–10% of human transitional-cell carcinomas (TCCs) of the bladder contain a mutated ras gene (Theodorescu et al., 1990). This raises the question of whether activated ras genes are causal to acquisition of the metastatic program of bladder tumors. Theodorescu et al. (1990) observed that the RT-4 human bladder carcinoma line is not invasive in nude mice, even after orthotopic injection of disaggregated cells. However, when a mutated human H-ras gene was transfected into RT-4 so that over-expression of the gene occurred in selected cell lines such as RT-4-mt-10 (RT-10), the selected cell line was able to locally invade the bladder after transurethral orthotopic inoculation of disaggregated cells. However, no contiguous or metastatic spread by RT-10 was found in other organs. The parental cell lines and the ras-transfectants all produced tumors when inoculated s.c. However, the tumors grew in the s.c. site as pseudoencapsulated masses without any evidence of tissue invasion (Theodorescu et al., 1990).

We have developed an intact-tissue implant method of orthotopic transplantation of human tumors in nude mice (Fu et al., 1991a,b). This method, first developed with colon cancer (Fu et al., 1991a), allows surgical patient tissue to be directly grafted onto the orthotopic organ of the nude mouse with resulting local growth and subsequent extensive regional metastases, lymph-node and liver metastases (Fu et al., 1991a). The method utilized harvested, s.c.-grown tissue of RT-10 as a tissue source, and showed that the orthotopically-implanted RT-10 was highly metastatic to many organs in the nude mouse (Fu et al., 1991b). These results contrasted with findings obtained by injection of disaggregated cells of RT-10, which were invasive but not metastatic (Theodorescu et al., 1990). We have now applied the orthotopic implant method to histologically intact tissue of the parental RT-4 itself and found that it, too, was highly metastatic.

RT-4 human bladder cells were obtained from the ATCC (Rockville, MD) and grown s.c. in 4-week-old athymic female nude mice. The s.c. grown tumors were excised and cut into 2-mm³ pieces and orthotopically implanted as previously described (Fu et al., 1991a,b). Briefly, the nude mice were anesthetized with isoﬂurane inhalation and the lower abdomen was sterilized with iodine and alcohol swabs. A small midline incision was made and the urinary bladder was exposed. The surgical adhesive 2-cyanoacrylate acid ester was applied on one side of the 2-mm³ tumor xenograft tissue and the piece of tumor was subsequently glued on top of the urinary bladder. The abdominal incision was closed with 7-0 silk surgical sutures in one layer. The animals were then kept in a sterile environment. When the animals were moribund they were sacrificed and full autopsies were performed. At autopsy all major organs were grossly examined. Each organ was fixed in 10% formalin, dehydrated, embedded in paraffin, sectioned and stained with hematoxylin and eosin.

After orthotopic transplantation of RT-4 to the nude-mouse bladder, extensive local growth occurred (over 2 cm × 2 cm) along with invasion of the bladder metastases to the lymph nodes, diaphragm, abdominal wall, omentum, pancreas and liver (Table I). These results are similar to those with the ras-H-transformed RT-4 tumor T1-10 after orthotopic implantation of intact tissue (Fu et al., 1991b). Figure 1a shows a nude mouse bladder in which RT-4 had grown orthotopically for 83 days. As can be seen, the tumor formed large masses in the abdominal area and became much larger than the bladder itself (also shown). Figure 1b shows the nude mouse bladder after orthotopic implantation of normal human-bladder tissue which did not result in tumor formation, thus emphasizing the tumor-forming ability of RT-4. Figure 1c shows the pathohistology of the locally-growing RT-4 tumor in the nude mouse which is a typical transitional-cell carcinoma. Figure 1d shows a greatly enlarged nude-mouse iliac lymph node involved with tumor. Figure 1e shows the pathohistology of the tumor-involved lymph node in the nude mouse, indicating typical transitional-cell carcinoma. Figure 1f shows the human RT-4 bladder tumor which has metastasized to the pancreas, liver serosa and lymph nodes in the nude mouse. The metastatic tumor growth on the liver serosa did not invade the parenchymal tissue. Therefore, the possibility of transcoclear spread cannot be ruled out. Figure 1g shows the histopathology of the pancreatic metastasis in the nude mouse. Figure 1h shows tumor-involved gastric lymph nodes. Figure 1i shows the pathohistology of the tumor-involved gastric lymph node of the nude mouse, indicating transitional-cell carcinoma. Figure 1j shows the excised nude mouse diaphragm fully involved with bladder tumor. Figure 1k shows the pathohistology of the tumor-involved diaphragm of the nude mouse indicating transitional-cell carcinoma. The entire diaphragm was replaced by

**Table I - Growth and Metastasis of the RT-4 Bladder Carcinoma in Nude Mice after Orthotopic Implantation**

<table>
<thead>
<tr>
<th>Implantation strategy</th>
<th>Mouse number</th>
<th>Primary tumor growth</th>
<th>Local invasion</th>
<th>Lymph-node metastasis</th>
<th>Organ metastasis</th>
</tr>
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<tbody>
<tr>
<td>Orthotopic implantation of intact RT-4 tumor tissue</td>
<td>8</td>
<td>8/8</td>
<td>8/8</td>
<td>5/8</td>
<td>3/8</td>
</tr>
<tr>
<td>Orthotopic implantation of intact normal human bladder specimen</td>
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</tr>
<tr>
<td>Orthotopic implantation of intact normal mouse bladder specimen</td>
<td>3</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
</tbody>
</table>

*Lymph nodes include: iliac lymph node, superficial inguinal lymph node, gastric lymph node, pancreatic lymph node.*

*Organ include: liver, pancreas, diaphragm, omentum.*
the metastatic growth, its normal structure being completely destroyed.

The spread of RT-4 to lymph nodes and other specific sites on a repeatable basis makes it unlikely that the distant tumor growth is due to non-specific seeding from the enplanted procedure. These results contrast with findings obtained when disaggregated RT-4 cells were injected transurethrally, in which case neither invasion nor metastases can be observed (Theodorescu et al., 1990). Similarly, when RT-4 was implanted s.c., only encapsulated tumors were formed (Theodorescu et al., 1990). The results, however, are consistent with the invasive behavior of the original tumor. cell--cell interactions are necessary for the full expression of metastatic potential in these tumors. The use of such a model may give a more realistic picture of the effects of individual genes on the metastatic program.

Thus, for bladder tumors and possibly others it may be critical for the tissue to remain histologically intact in the orthotopic xenografting process so that its metastatic potential may be fully expressed. Thus, it is quite possible that native cell--cell interactions are necessary for the full expression of metastatic potential in these tumors. The use of such a model may give a more realistic picture of the effects of individual genes on the metastatic program.

Yours sincerely,

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REFERENCES


